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TOXICOLOGY.—*Toxicity of some dinitrophenols to the American dog tick*, Dermacentor variabilis (Say).[1] OSCAR E. TAUBER, ANNE HAGER TAUBER,[2] CHARLES R. JOYCE,[2] and WILLIS N. BRUCE. (Communicated by CARL J. DRAKE.)

Pastac (11) indicates, without reference or date, that the first notice of the value of a nitro dye as an insecticide came through the observation that clothes moths did not molest wool dyed with martius yellow (dinitronaphthol). In the past 30 years a considerable number of laboratory and field tests with many dinitrophenols have been conducted on a number of different insects (3, 7, 8, 8a, 9, 9a, 10, 11, 12, 13, 14, 15). Some of these same dinitro compounds have been recommended as weedkillers, fungicides, etc. (2, 6, 11, 17). No records of the effects of any dinitrophenols on ticks have been found.

Ticks are particularly concerned in the transmission of relapsing fever and typhus-like diseases. Recently (1942) Anigstein and Bader (1) reported evidence suggesting *Amblyomma americanum* as an additional carrier of Rocky Mountain spotted fever. At this writing, when military training and actual warfare bring many thousands of men into possible contact with various potentially dangerous Ixodidae, any suggestions that may contribute to methods for extermination of ticks should prove timely.

Ticks are very tenacious of life. Past attempts at control and eradication have involved such laborious procedures as mechanical removal by handpicking or entanglement in sheep wool; dipping domestic animals; cutting or burning tick infested brush; or by trapping, poisoning, or shooting of hosts other than man and domestic animals. Since these hosts sometimes also include such active forms as bats and birds, the last three of the enumerated methods of eradication are hardly possible or efficient. Also, elimination of rodents or other wild hosts over a large area is likely to upset some biologic balance and initiate new problems of another nature.

The experiments to be described were begun as preliminary ground-work for contemplated field trials to kill ticks in selected areas by dusting vegetation in which they are concentrated. Such dustings might reach the ticks directly as they rested on the vegetation or crawled on the ground. It might reach them when their wild hosts, such as mammals and ground-feeding birds, moved through the dusted herbage and brushed and shook the toxic material on themselves and their parasites. So far as we can determine, this proposed approach is a new attack on the tick problem.

Should this dusting of vegetation prove of value in killing ticks, such a technique might be useful in reducing populations of mosquitoes, chiggers, certain flies, and other forms that rest on herbage, or wait for victims while hanging on grass, shrubs, or other plants. The method might conceivably be the answer to ridding jungle trails of blood-sucking land leeches, which are a real

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menace to travelers and soldiers in Indo-China, Malaya, and other areas of that region where rainfall is especially abundant. "Blanket" dusting of refuse piles and dumps may also serve to bring the toxic dusts to fleas and lice carried by rats.

With these and other ideas of control in mind, preliminary tests on fleas and other pests have been inaugurated to ascertain killing doses before field work is begun. These latter results will be published in subsequent papers.

CHEMICALS

Whenever possible, toxic compounds were obtained as pure chemicals and diluted as desired in the laboratory. To insure thorough dispersal, weighed ingredients were first mixed by spatula on a glass plate, then shaken in a large jar, and last placed on a home-made "roller-ball-mill" by which a cylinder, containing the mixture and pebbles or glass marbles, was rolled over and over for several hours (see Fig. 1). Among the more promising toxic materials were dinitro-ortho-cresol (DN-o-C), supplied by Standard Agricultural Chemicals, Inc.; dinitro-ortho-cyclohexyl-phenol from Dow Chemical Co.; and ammonium-dinitro-ortho-cresylate and guanidine dinitro-ortho-cresylate from American Cyanamid & Chemical Corporation. Diluents included 320 mesh sulphur from Stauffer Chemical Co.; "Pyrophyllite" from E. I. du Pont de Nemours & Co.; and "Pyrite" from Dow. Other compounds, such as sodium arsenite, were pure chemicals available from laboratory stock.

The outstanding toxicity of 3,5-dinitro-ortho-cresol, as demonstrated by Decker and Drake (3), when compared with 24 other dinitro compounds, was the incentive for using the DN-o-C as the main toxic agent when these investigations were begun. Preliminary tests with other compounds were inserted in the program as the chemicals became available from the manufacturers.

There is some disagreement regarding the correct naming of the dinitro-ortho-cresol. Insect toxicologists generally refer to it as the 3,5-compound, but Filbert (5), of du Pont de Nemours & Co., states that 4,6-dinitro-o-cresol is the correct numbering as approved both by Chemical Abstracts and Beilstein.

EXPERIMENTAL ANIMALS

Dermacentor variabilis (Say) is a widely distributed American dog tick. It is implicated in transmission and dispersal of Rocky Mountain spotted fever. The number of infested specimens usually runs from 1 in 200 to 1 in 600 (16). In certain areas of Iowa it has sometimes been numerous enough (4) to be a potentially dangerous carrier of spotted fever to human beings.

Adult specimens of *D. variabilis* were collected by hand from dogs, or by "flagging" in those localities of Iowa where ticks of this species were known to be numerous. Ticks thus obtained were kept over moist sand in cotton-stoppered vials. These collections included ticks of all ages within their adult life span. Also, individuals ranged through all stages of nutrition, including full engorgement, interrupted feeding, and starvation. In some cases the period of starvation may have been for more than a year. In addition, the collections sometimes included spent females, or gravid females that began to oviposit while under observation as control or experimental subjects. In short, these adult ticks were of wild stock and possessed both the good and bad characteristics of an heterogeneous population.

Larval ticks were hatched from eggs deposited in the laboratory by females taken in the field. Larvae of a known age were thus available for tests. Other larvae were allowed to feed on white-footed mice, *Peromyscus leucopus noveboracensis* (Fischer), and, after transformation to nymphs, established a source of nymphs of known age.

Egg masses were collected in the laboratory, and tests were made of some of the dinitrophenols as tick ovicides.

METHODS

Not the least of the problems this investigation involved was that of devising some technique of bringing the ticks and the compounds together in a simple procedure that could be easily and reasonably duplicated. After various trials, the following set-up

and technique were employed for adult ticks: A circular opening, 6 inches in diameter, was cut in a piece of cardboard resting on a sheet of paper toweling. A 2-inch disk of cardboard was placed in the center of the 6-inch opening. Over the opening was placed a dusting tower consisting of a tall bell jar with an opening near the bottom, through which a dusting nozzle could be inserted (see Fig. 1). Known weights of dust were pumped into the tower while the nozzle was shifted about, inside the apparatus, to insure as even a distribution of dust as possible. After the dust settled, the tower was lifted away, and the entire cardboard pattern was removed. A 2-inch circular band of dust was thus formed on the toweling. Ticks to be tested were placed in the central dust-free area, and then recaptured outside the dust ring after voluntarily walking across it to the outer dust-free area.

With nymphal ticks, the band of dust was reduced to 1 inch by merely using a 4-inch disk to make the inner dust-free surface. Younger larval ticks were so small and "bogged down" so easily in the dust ring that a further modification was necessary. For all larvae, therefore, the following uniform procedure was used. A small nontoxic dust ring of pyrophyllite was first set up. The test larvae were set free inside this ring. The dust tower was then set in place and the dust blown in. When the tower was removed the dusted larvae were picked up and then confined to vials.

Adult ticks and larger nymphs were easily handled with tweezers. Larval ticks were moved about on the pointed tip of a moistened brush.

After treatment, adult and nymphal ticks were confined separately in small vials and examined at regular, convenient intervals. Death of the specimen was recorded when no movement whatsoever was elicited even in the close approach to warmth from a light bulb. Larval ticks were usually kept in groups of 5 or 10 individuals to the vial.

All untreated controls were kept in the same type of container and under the same conditions as the treated ticks.

Actual determinations of the weight of dust distributed in the ring under the dust tower gave a quantity equivalent to about 65 to 75 pounds an acre for the adult and nymph treatments. For larvae, the quantity was about 20 to 25 pounds an acre.

RESULTS AND DISCUSSION

All results presented throughout this paper represent data collected under controlled laboratory conditions. Under no circumstances are they to be construed as results to be expected with field trials. It was the intention to carry on field operations during 1942, but by the time these preliminary laboratory tests were completed, the season suitable for outdoor tests was too far advanced. Since these present results may be of value to other workers who could make field runs before we can in the summer of 1943, our data are presented now. In any event, field trials will be conducted in Iowa in 1943 if the necessary equipment and labor can be assembled.

Fig. 1.—In the background is the tall bell jar used as a dusting tower. A charge of dust is suspended in its interior. In the foreground is the mixing-mill used to roll the dust mixtures.

Although test specimens were often kept under observation for a week or more, and controls were checked for several weeks at least, only the 24- and 48-hour mortality percentages are presented here. From the standpoint of toxicological interest, the 24- and 48-hour results are probably of most significance. Beyond 48 hours other factors than the exposure to the test dust are likely to come into play. Also, if a tick is a vector of a disease, the faster it is eliminated the better, if no other complications are involved.

Adult Dog Ticks

One of the first facts that became clearly evident in the results was the difference in resistance to DN-o-C between unfed and engorged adult ticks. This characteristic is demonstrated in the sample of data presented in Table 1.

TABLE 1.—MORTALITY OF UNFED AND ENGORGED ADULT DOG TICKS (*Dermacentor variabilis*) AFTER CONTACT WITH DINITRO-O-CRESOL DILUTED WITH PYROPHYLLITE

Nutritional state	DN-o-C	Number tested	Dead at 24 hours	Dead at 48 hours
	Percent		*Percent*[1]	*Percent*[1]
Unfed.....	2	20	35	35
Unfed.....	4	20	40	45
Unfed.....	8	50	64	68
Unfed.....	12	50	72	88
Engorged....	8	50	36	36
Engorged....	12	50	45	45

[1] Throughout this entire paper, percent of mortality is expressed in the nearest whole number.

Additional evidence that the nutritional state of these ticks is an important consideration was demonstrated in the summary of mortality of specimens of this species kept as controls under laboratory conditions. This summary is given in Table 2.

TABLE 2.—MORTALITY OF ADULT CONTROL SPECIMENS OF *Dermacentor variabilis*

Nutritional state	Number observed	Dead at 24 hours	Dead at 48 hours
		Percent	*Percent*
Unfed.....	180	11	17
Engorged.....	65	3	4

The high mortality of the unfed individuals is rather striking, and no explanation can be offered. Unfavorable humidity is probably a factor under laboratory conditions, even though some efforts were made to keep the test ticks from dehydration. So little is known regarding certain limiting ecological factors in the tick's life history that some of our colony-maintenance procedures were probably faulty. Under the pressure of present conditions, however, it was decided not to take time to explore these rearing problems, but to proceed to the more important toxicological aspects.

In regard to the high mortality, difference in the nutritional conditions is naturally the first suggested clue, but more complicated relationships may be involved. No attempt was made to check the life span of individuals under field conditions, but there is no reason to assume that such a high mortality among adults is a natural one. When one considers, also, that most unfed ticks were collected by the "flagging" method, which entails only slight chances of injury, while the engorged specimens were often dislodged with considerable difficulty from the skin of their hosts, the difference in mortality of the two types is even less easily explained. Nevertheless, in spite of the high death rate of unfed controls, the data of Table 1 show a good gradient of effect through the use of increased strengths of the dinitro-o-cresol.

Just what parts body surface and body volume, considered separately or together, might have in effecting the difference in mortality of unfed and engorged ticks is also unknown. Engorged ticks generally picked up considerably more of the chemical while walking through the band of dust. However, this factor of actual greater contact by swollen engorged ticks apparently was not sufficient to counteract the relatively larger amount of dust which the unfed ticks acquired. The ratio of body surface to body volume would, of course, be higher in the unfed ticks. The smaller, unengorged specimens could thus acquire a higher internal concentration of the absorbed poison, even though the actual contact was less.

TABLE 3.—MORTALITY OF UNFED AND ENGORGED ADULT DOG TICKS AFTER CONTACT WITH SULPHUR-DILUTED DINITRO-ORTHO-CRESOL

Nutritional state	DN-o-C	Number tested	Dead at 24 hours	Dead at 48 hours
	Percent		*Percent*	*Percent*
Unfed.....	4	100	57	65
Unfed.....	8	50	73	77
Unfed.....	12	50	60	68
Unfed.....	20	135	91	94
Unfed.....	25	110	87	98
Engorged...	4	25	16	28
Engorged...	8	50	42	60
Engorged...	12	25	47	80
Engorged...	25	70	51	96

When ordinary 320-mesh dusting sulphur was substituted for pyrophyllite as a diluent for the dinitro-ortho-cresol, a rather well-

marked general trend of increased toxicity was often noted, especially in the lower concentrations of DN-o-C. These data are given in Table 3 (compare with Table 1).

Tests with 100 per cent, 320-mesh sulphur in the 2-inch circular band of dust served to emphasize again the difference in susceptibility of unfed and engorged ticks. This information is found in Table 4.

TABLE 4.—TOXICITY OF 100 PERCENT SULPHUR (320 MESH) TO THE ADULT DOG TICK

Nutritional state	Number tested	Dead at 24 hours	Dead at 48 hours
		Percent	*Percent*
Unfed	120	19	27
Engorged	25	3	3

Weather conditions at, and transportation difficulties to, the usual sites of collections sometimes made it impossible to obtain, at the right time, as large samples as were desired for tests. With larger numbers of individuals, the discrepancy in the 8 per cent and 12 per cent trials with unfed specimens of Table 3 might be eliminated. The small sample and the heterogeneous character of the field-collected ticks may also account for the lower mortality among the few engorged ticks tested with 100 per cent sulphur than among the larger sample of control ticks in Table 2. Comparison of per cent mortalities in Tables 1 and 3 shows, however, that the use of sulphur as a diluent is a valuable procedure, especially with unfed ticks. Similar trials with 100 per cent pyrophyllite showed no mortality percentage above that found for the controls.

No attempt was made to set up experiments to test for synergistic action in the sulphur and dinitro-ortho-cresol mixtures. Such tests are planned when next season's ticks become available.

Just before the 1942 tests had to be terminated because of increased seasonal difficulties in obtaining ticks, several other compounds were received from manufacturers, and preliminary tests were run with the few ticks then available. One of these chemicals, the ammonium dinitro-ortho-cresylate, has shown excellent promise with certain insects (8a) and other near relatives of ticks. Results from these compounds are in Table 5. Also included in this table are data from the use of sodium arsenite at 4 per cent and 100 per cent levels. These arsenite tests were included merely as reference and comparison points with a more familiar toxic dusting compound.

TABLE 5.—PRELIMINARY RESULTS WITH MISCELLANEOUS COMPOUNDS USED AS DUSTS ON UNFED ADULT DOG TICKS

Compound	Per-cent	Diluent	Number tested	Dead at 24 hours	Dead at 48 hours
				Percent	*Percent*
$NaAs_2O_3$	4	Pyrophyllite	25	8	20
$NaAs_2O_3$	100	—	25	64	92
Guanidine dinitro-o-cresylate	12	Sulphur	30	0	23
Ammonium dinitro-o-cresylate	12	Sulphur	30	87	94

Tables 1, 3, and 5 offer the opportunity to compare the toxicity of several of the compounds tested on unfed dog ticks. At the 12 per cent level, the ammonium dinitro-ortho-cresylate seems the most toxic of the tested materials. At the 4 per cent levels, the sodium arsenite has about half the mortality per cent of dinitro-ortho-cresol; and, when the latter was combined with sulphur, a 25 per cent concentration had approximately the same toxicity for unfed specimens as 100 per cent sodium arsenite. Guanidine dinitro-ortho-cresylate was the least toxic of the chemicals tried on adult dog ticks.

TABLE 6.—MORTALITY OF NYMPHAL DOG TICKS AFTER CONTACT WITH VARIOUS DN-COMPOUNDS DILUTED WITH SULPHUR

Age	Compound	Per-cent	Number tested	Dead at 24 hours	Dead at 48 hours
				Percent	*Percent*
5 days	DN-o-C	8	30	50	57
5 days	DN-o-C	12	35	63	66
5 days	DN-o-C	16	50	74	96
2 weeks	DN-o-C	8	50	62	72
2 weeks	DN-o-C	12	50	96	98
2 weeks	DN-o-C	16	30	100	—
3 weeks	DN-o-C	8	30	97	97
3 weeks	DN-o-C	12	75	98	98
3 weeks	DN-o-C	16	50	100	—
3 weeks	Guanidine-DN-o-cresylate	12	30	33	60
3 weeks	Ammonium-DN-o-cresylate	12	80	100 (in 3½ hrs.)	—

Nymphal Dog Ticks

As stated previously, some larval ticks were allowed to feed in the laboratory on caged wild white-footed mice, and then used after transformation to the nymphal stage. All the data secured from tests on nymphs are set up in Table 6.

Several series of untreated, control nymphs were set up at the same time. Their data appear in Table 7.

TABLE 7.—MORTALITY OF UNTREATED NYMPHAL DOG TICKS

Age	Number observed	Dead at 24 hours	Dead at 48 hours
		Percent	*Percent*
5 days	30	0	0
2 weeks	50	4	22
3 weeks	30	13	28

One of the first facts apparent from Tables 6 and 7 is the decreased vigor of the nymphs as they become older. This point is demonstrated not only in the increased mortality of the controls, but also in the greater susceptibility to treatment with poisons. Table 6 also shows a regular progressive build-up in toxicity as the percentage of dinitro-o-cresol is increased. All nymphs were laboratory reared and were thus a stock of more nearly homogeneous test animals whose history was better known than that of the field-collected adults. Consequently, discrepancies in resultant data are not so likely to occur because of differences in age, nutrition, and other factors.

In addition, Table 6 makes it clear that of the two tested cresylates the guanidine compound is decidedly inferior to the ammonium dinitro-o-cresylate, and the latter is superior to the dinitro-o-cresol. While the 12 per cent dinitro-o-cresol attained 98 per cent mortality in 48 hours, the 12 per cent ammonium dinitro-o-cresylate brought about 100 per cent mortality in only three and a half hours.

TABLE 8.—MORTALITY OF 5-DAYS, 2-WEEKS AND 3-WEEKS OLD LARVAL DOG TICKS AFTER DUSTING WITH DINITRO-ORTHO-CRESOL DILUTED WITH PYROPHYLLITE

Age	Concentration of DN-o-C	Number tested	Dead at 1 hour	3 hours	6 hours	18 hours	24 hours	48 hours
	Percent		*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
5 days	0.063	30	33	87	100			
5 days	0.125	30	37	100				
5 days	0.25	30	100					
5 days	0.5	30	100[1]					
5 days	1.0	30	100[1]					
5 days	2.0	30	100[2]					
2 weeks	0.063	30	—	—	—	—	0	0
2 weeks	0.125	30	—	7	—	—	23	33
2 weeks	0.25	30	—	33	—	—	67	80
2 weeks	0.5	30	—	40	—	—	72	88
2 weeks	1.0	30	—	63	70	—	80	90
2 weeks	2.0	30	76	—	93	—	100	
3 weeks	0.5	30	—	13	33	37	37	37
3 weeks	1.0	30	47	—	67	70	70	73
3 weeks	2.0	30	57	—	70	73	73	77
3 weeks	4.0	30	100					

[1] In 55 minutes.
[2] In 20 minutes.

Larval Dog Ticks

A large supply of larval ticks made it possible to make runs through a longer series of concentrations at various ages of the test species. Trials were run at the following ages: 5 days, 2 weeks, 3 weeks, and 4 weeks. The first three ages were tested only with dinitro-o-cresol diluted with pyrophyllite. The 4-weeks larvae were tested after it was found that sulphur made a better diluent than pyrophyllite, and, unfortunately, it was not then possible to repeat the previous tests on younger larvae, using sulphur. Also, when the 4-weeks larvae were available, certain other chemicals were received and these were also tried. And, again, to our regret, it was not possible, last season, to use these latter compounds on other stages of ticks. Their data, however, are included as a matter of record of preliminary trials. Results on larval ticks will be found in the next two tables; a dash (—) in the body of the tables indicates that no check count for mortality was made at that particular time interval.

Contrary to the situation in nymphs, which seem to be less hardy with increasing age, Tables 8 and 9 show that larval ticks become more resistant as they get older. For example, at the 2 per cent level of dinitro-o-cresol the youngest larvae (5 days old) were all dead within 20 minutes; the 2-weeks specimens were all dead at 24 hours; and the three-weeks larvae had a mortality of 77 per cent at 48 hours. At 4 weeks of age, resistance increased to the point where 8 per

TABLE 9.—MORTALITY OF 4-WEEKS OLD LARVAL DOG TICKS AFTER DUSTING WITH VARIOUS COMPOUNDS

Compound	Concentration	Diluent	Number tested	Dead at 1 hour	Dead at 3 hours	Dead at 6 hours	Dead at 12 hours	Dead at 24 hours	Dead at 48 hours
	Percent			*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
DN-o-cresol	4	Sulphur	30	3	—	6	30	50	63
DN-o-cresol	8	Sulphur	30	10	67	90	—	100	
DN-o-cresol	12	Sulphur	30	13	97	100[1]			
DN-o-cresol	8	Pyrophyllite	30	80	100[2]				
DN-o-cresol	12	Pyrophyllite	30	97	100[3]				
DN-o-2nd butylphenol	8	Pyrophyllite	30	97	100[4]				
DN-o-cyclo-hexylphenol	8	Pyrophyllite	30	67	80	100[5]			
Dicyclohexyl-amine salt of DN-o-cyclo-hexylphenol	20	Pyrophyllite	30	3	20	—	57	100	
Dinitroso-resorcinol	100	—	30	3	6	—	100		
Tetrachlorophenol	100	—	30	33	100				
Pentachlorophenol	100	—	30	0	77	—	100		
Hexachlorophenol	100	—	30	6	57	—	100		
Sodium arsenite	4	Pyrophyllite	30	—	10	—	17	17	27
Sodium arsenite	100	—	30	73	87	100			

[1] In 5½ hours. [2] In 2½ hours. [3] In 1½ hours. [4] In 4½ hours. [5] In 8½ hours.

cent dinitro-o-cresol was necessary to give 100 per cent kill in 2½ hours; and with 12 per cent, 100 per cent mortality in 1½ hours.

Of the four other dinitrophenols listed in Table 9, two (the secondary butylphenol and the cyclo-hexylphenol) give promise of being as toxic as the dinitro-ortho-cresol, all tested at the 8 per cent level. The other two compounds (the amine salt and the resorcinol) were tested at much higher concentrations (20 per cent and 100 per cent, respectively) and showed no more toxicity for larval ticks than the 8 per cent DN-o-C.

All three of the -chlorophenols were tried without dilution, and, even at 100 per cent concentration, they were no more effective than DN-o-C in the range of 8 per cent and 12 per cent levels.

Two widely separated concentrations (4 per cent and 100 per cent) of sodium arsenite were tested on 4-weeks larvae, and just as in the case of unfed adult ticks (see Table 5), were considerably less effective than comparable percentages of dinitro-ortho-cresol.

The pronounced fragility of the young larval ticks is re-emphasized by the mortality data of controls, shown in Table 10. In this case, the high death rate of young larval ticks is probably a reflection of what occurs in nature also. When one compares

TABLE 10.—MORTALITY OF UNTREATED CONTROL LARVAL TICKS

Age	Number observed	Dead at 24 hours	Dead at 48 hours
		Percent	*Percent*
5 days	50	13	27
2 weeks	50	0	0
3 weeks	50	0	0
4 weeks	50	0	0

the large number of eggs, which each female tick produces, with the smaller number of ticks which reach maturity, it is evident that there must be some phase of post-hatching development during which survival is difficult. Toxic dust treatments may be able to utilize the lethal possibilities of this critical period.

Dog Tick Eggs

Two experiments were set up to test DN-o-C as a tick ovicide.

In the first test 15 clumps of eggs were placed under a bell jar and dusted with 12 per cent DN-o-C at the rate of about 50 pounds/acre. There was no noticeable decrease in hatching, after the usual incubation period.

In the second test 10 clumps of eggs were dusted in the same manner but with 25 per cent DN-o-C. After a sufficient incubation

period elapsed, the clumps were examined. There was an obvious reduction in the number of eggs that hatched, in comparison with undusted control clumps kept under the same laboratory conditions. Eggs at the bottom of the dusted clumps, those eggs not directly in contact with the 25 per cent DN-o-C, were the only ones which produced young ticks. Those dusted eggs on top and at the sides evidently were killed.

If these two rough tests are of any significance, they indicate a considerable resistance to toxic substances by tick eggs. In both of the above tests the dusts remained in contact with the eggs throughout the entire incubation time. Dusts applied under field conditions would probably not remain so closely applied during approximately three weeks of weathering. Attempts at eradication or decrease of ticks by dusting the eggs would probably not be practicable. It appears that the egg stage is not the tick's most vulnerable period.

CONCLUSIONS

On the basis of laboratory tests alone, the following statements are presented.

1. Unfed and engorged adult specimens of the American dog tick, *Dermacentor variabilis*, possess a decided difference in susceptibility to contact with dinitro-ortho-cresol and other dinitrophenols. For example, 12 per cent DN-o-C, with pyrophyllite as a diluent, applied at the rate of 65 to 75 pounds to the acre, has a 48-hour mortality of 88 per cent with unfed adults; and 45 per cent with engorged adults.

2. The use of 320-mesh dusting sulphur as a diluent, in combination with DN-o-C, makes a more toxic mixture against ticks than that obtained with pyrophyllite as the diluent. With 8 per cent DN-o-C, at 48 hours, the per cent of mortality for unfed adults is 68 per cent with pyrophyllite; 77 per cent with sulphur. For engorged adults, with 8 per cent DN-o-C, the per cent dead is 36 with pyrophyllite and 60 with sulphur.

3. Sulphur alone has some toxicity for unfed adult dog ticks. Applied at the rate of 65 to 75 pounds an acre, 100 per cent sulphur killed 19 per cent unfed ticks in 24 hours. It had no effect on the particular sample of 25 engorged specimens tested in the same manner.

4. Even in combination with sulphur, and at 65 to 75 pounds an acre, DN-o-C mixtures must contain at least 25 per cent of the DN compound to produce a kill over 95 per cent within 48 hours. The adult dog tick is tenacious of life.

5. Ammonium dinitro-o-cresylate gives promise of higher toxicity than DN-o-C. In preliminary tests, a 12 per cent concentration with sulphur is nearly equal in toxicity to 25 per cent DN-o-C when applied in identical dosages.

6. Undiluted sodium arsenite is slightly less toxic to unfed adult dog ticks than DN-o-C diluted at 25 per cent with sulphur, when applied in identical dosages, with identical technique.

7. Guanidine dinitro-ortho-cresylate does not show much promise as a tickicide.

8. Nymphal dog ticks decrease in vigor as they age during the nymphal stage. This is shown both by increased mortalities among untreated controls, and by greater susceptibility to dusting with DN compounds.

9. At 65 to 75 pounds an acre, a sulphur and DN-o-C mixture must contain at least 16 per cent of the cresol to kill more than 95 per cent of the younger (5 days old) nymphs within 48 hours. A 12 per cent DN-o-C will kill more than 95 per cent of 2 to 3 weeks old nymphs within 24 hours; 16 per cent kills 100 per cent in less than 24 hours.

10. With nymphal ticks ammonium DN-o-cresylate again shows superior toxicity; 12 per cent in sulphur kills 100 per cent of 3-weeks nymphs in $3\frac{1}{2}$ hours.

11. Larval dog ticks become more hardy with age. For example, when treated with 2 per cent DN-o-C, 5 days old larvae were all dead in 20 minutes; the 2-weeks specimens were all dead at 24 hours; and 3-weeks larvae had a mortality of 77 per cent at 48 hours. At 4 weeks of age the DN-o-C concentration had to go to 8 per cent to kill 100 per cent in 24 hours.

12. When tested with 4-weeks old larval ticks, dinitro-o-secondary butylphenol and DN-o-cyclohexylphenol appear nearly as toxic as DN-o-C. The dicyclohexylamine

salt of DN-o-cyclohexylphenol is decidedly less toxic than DN-o-C.

13. Dinitrosoresorcinol, and the tetra-, penta-, and hexa-chlorophenols seem to have little value as tick larvicides.

14. Dog-tick eggs are quite resistant to poisoning by DN-o-C. When dusted with 12 per cent DN-o-C, no noticeable reduction in hatching occurred. Dusting with 25 per cent DN-o-C killed those eggs with which it came into direct contact, and on which it stayed during the entire incubation period.

15. With the above results in mind it seems an inevitable conclusion that field control of the American dog tick probably will be a difficult, but not impossible, problem if attacked with DN-o-C or NH_4-DN-o-cresylate. There seems to be no particularly vulnerable spot during its life history. It is most easily killed during early larval life, but that susceptibility does not help much, for practical purposes, since hatching occurs over a long period during warm weather. Only repeated dusting over several months could take advantage of this weakness.

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